University of Cincinnati

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I, Rebecca Moore , hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:

The relationship between a dietary pattern high in fruits, vegetables, low fat dairy, and whole grains and low in red meat and vascular structure and function in individuals with type 2 diabetes

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The relationship between a dietary pattern high in fruits, vegetables, low fat dairy, and whole grains and low in red meat and vascular structure and function in individuals with type 2 diabetes

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

Master of Science

In the Department of Nutrition of the College of Allied Health Sciences

By

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Abstract

Diet has been shown to impact vascular structure and function in diverse populations[3,4,9,16,17,18,21], however little is known about the impact of the Dietary Approaches to Stop Hypertension (DASH) dietary pattern on the vasculature. The DASH dietary pattern is high in fruits, vegetables, low fat dairy foods, and whole grains and is low in red meat, fats and oils, and sweets. Recent research suggests that this dietary pattern may have beneficial effects on blood pressure, blood lipids and inflammation, which may in turn positively impact vascular structure and function. The present study is a cross-sectional secondary data analysis from a study originally designed to examine the effects of obesity and type 2 diabetes mellitus (T2DM) on vascular health. Specifically, this study examined the association between DASH dietary adherence as measured by 3-day dietary recall and calculated DASH score index and carotid intima media thickness (cIMT), augmentation index (AI), pulse wave velocity (PWV) , and brachial distensibility (BrachD) in young adults with T2DM. The participants were diagnosed with T2DM with an average disease duration of 8 years and had, on average, poorly controlled blood glucose and low DASH dietary adherence. There were no significant associations between DASH dietary adherence and vascular structure and function measurements in this population of young adults with T2DM. This finding suggests that cardiovascular benefits of the DASH diet appear to be independent of peripheral mechanisms in young adults with T2DM.

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Introduction

Cardiovascular disease (CVD) is an expensive and prevalent condition, taking nearly 600,000 lives each year and those with T2DM are at high risk for developing CVD[9]. Diet has a significant impact on overall health and well-being; however little is known about the impact of diet on vascular structure and function. This study aims to examine the association between a dietary pattern that is high in fruits, vegetables, low-fat dairy foods, whole grains and low in red meat, fats and sweets, also known as a Dietary Approaches to Stop Hypertension (DASH) dietary pattern[17] and vascular structure and function in individuals with T2DM. Empirical evidence suggests that blood lipid levels, endothelial function, CVD and diabetes-related risk factors are improved after following certain dietary patterns. For example, diets high in fiber have been shown to reduce arterial stiffness as well as improve vascular function[37]. Diets high in fruits and vegetables (over 6 servings a day) have been shown to improve blood lipid levels and endothelial function[13, 22]. Low fat dairy foods, despite containing some saturated fat, are related to improved vascular function[4, 24, 25]. Whole grain foods as a source for fiber and other important vitamins and minerals have a similar beneficial effect[40]. Diets high in proteins and fats from fish and legumes have also been shown to improve vascular outcomes[12, 13, 16, 19]. Given evidence supporting a beneficial relationship between diets high in fruits, vegetables, low fat dairy, and whole grains and low in red meat and vascular health, it stands to reason that greater adherence to a DASH-type dietary pattern, which is high in all of these food groups, would be positively related to vascular structure and function in T2DM. This study will examine this hypothesis; specifically, there will be a positive relationship between level of adherence to a DASH-style diet and vascular structure and function in individuals with T2DM.

Review of Literature

A. Diabetes and Vascular Health

Cardiovascular disease (CVD) is multifaceted and can occur as a result of many risk factors including elevated blood lipids, diabetes mellitus, high blood pressure (BP), smoking, poor diet, high fasting glucose, and heredity. Approximately 49% of American adults have at least one risk factor for CVD and one in every three adults has hypertension (BP at or above 140/90mmHg or 130/80mmHg with diabetes) and only about half have it under control. Only one third of the population is considered to be normotensive[9]. In 2009, hypertension alone resulted in 348,000 deaths and costs to the nation annually are estimated to be nearly $47.5 billion in procedure costs, medications, and missed days of work. Thus, there is a financial and nationwide health motive for reducing high BP. Due to oxidative stress and elevated blood lipids associated with diabetes, those with diabetes are at an even greater risk of suffering from adverse consequences including damage to the structure and function of the heart and vasculature. Diabetes is a growing epidemic in the United States, with 9.3% of the population diagnosed with this health problem. This translates to 21 million people diagnosed with diabetes in the United States. These numbers are expected to continue to rise, as are the cardiovascular events associated with diabetes. Between 2009 and 2012, of the adults with diabetes, 71% had hypertension and 65% had high LDL cholesterol[9], two of the three main risk factors for CVD. Notably, cardiovascular death rates are 1.7 times higher in those with diabetes when compared to those without diabetes[9]. Approaches to improving vascular health in individuals with diabetes are important because many of the risk factors for CVD are preventable and avoidable.

B. Measures of Vascular Health

Vascular health relates to the ability of blood vessels to transport blood and bodily fluids throughout the body. There are several ways to measure vascular health. Measures of arterial stiffness including augmentation index (AI), pulse wave velocity (PWV), and brachial artery distensibility (BrachD) measure how well the blood vessels dilate and contract in response to changes in blood volume and blood pressure[5, 15, 33, 34]. CVD risk factors such as hypertension, dyslipidemia and obesity have been shown to increase arterial stiffness. Therefore, this measure may be used in treatment. AI is calculated from a pulse wave analysis of blood flow and equals the ratio of late versus early systole in the pulse wave. During systole, the aorta dilates and the velocity at which the wave moves through the aorta is indicative of arterial compliance[31]. Individuals with type 2 diabetes (T2DM) have been shown to have an increased AI measure, indicating increased arterial stiffness. PWV is the rate at which a pulse wave travels from one point to another in the blood vessel. Elevated PWV has been shown to be a reliable measure used to predict cardiovascular morbidity and mortality in diverse populations, including individuals with T2DM[5, 33]. BrachD is a measure of the compliance of the brachial artery based on brachial artery pressure curves, which tends to decrease in the presence of hypertension[15]. Individuals with T2DM are at risk for vascular stiffness as measured by elevated AI and PWV and reduced BrachD; therefore these measures are important diagnostic tools for assessing vascular health.

An increase in thickness of the blood vessel is a structural measure of the vasculature that is related to arterial stiffness and poor vessel compliance. One of the most frequently used diagnostic measures of vascular structure is ultrasound assessment of the carotid intima media thickness (cIMT). Using this technique, an image of the carotid artery is most commonly taken from three segments of the artery (common, bifurcation (bulb), and internal)[33]. The common carotid measurement is taken close to the aorta, before the bifurcation (or splitting) of the vessel into the external and internal carotid arteries. Determination of arterial wall thickness is generally measured from the leading edge of the lumina-intima to the leading edge of the media-adventitia layers[34]. It has been shown that high blood pressure and T2DM are related to thicker carotid intima media and that a thicker cIMT is related to stiffer blood vessels[33]. While cIMT is most often used in research rather than clinical settings, it is nonetheless an important tool in predicting and preventing cardiovascular events.

C. Relationship between Diet and Vascular Health

The DASH dietary pattern was originally formulated as a combination of foods that optimized levels of key nutrients that showed blood pressure lowering potential. The diet was intended to lower blood pressure and improve blood lipid levels in patients at high risk of CVD[15]. Recent research suggests that the foods emphasized in the DASH dietary pattern may have the potential to not only lower risk factors for CVD, but directly reduce the damage to target organs adversely affected by CVD risk factors, e.g., blood vessels[7, 13, 15, 30, 33]. The DASH dietary pattern emphasizes fruits, vegetables, low-fat dairy, and whole grains as well as low intake of red meat. It also encourages fish, poultry, beans, seeds, nuts, and vegetable oils to provide healthy fats and protein. The diet is low in sodium (<2g/day) and low in added sugar. Overall, the diet emphasizes a nutrient composition that is high in potassium, calcium, magnesium, and fiber, moderate in protein and low in sodium, saturated, and trans fatty acids[17].

Blood vessel dilation is critical for blood pressure control and blood flow. The compound nitric oxide (NO) is released from the vascular endothelium to promote vasodilation. While the mechanisms by which the DASH diet contributes to blood pressure lowering have not been elucidated, increased production of NO may be one factor at play. Nutrient components of the DASH dietary pattern promote NO production, and thereby could promote vasodilation and vessel relaxation[22]. Fruits and vegetables, particularly leafy green vegetables, are high in inorganic nitrate which serves as a substrate for NO production and dietary nitrate accounts for about half of the NO concentration in the body[7]. Men who consume an extra 2.4 portions of fruits and vegetables a day while maintaining a dietary pattern similar to DASH have been shown to have higher microvascular reactivity[22]. It has been proposed that the flavonoids in fruits and vegetables also improve NO availability by increasing gene expression of the enzyme responsible for producing NO or by increasing the availability of the substrate l-arginine[22]. Sodium intake has been shown to reduce NO production, thereby impairing vasodilation[2]. NO production is a strong indicator of endothelial function and can be used as a diagnostic tool for vascular function indirectly via the assessment technique flow mediated dilation (FMD)[15]. FMD measures the ability of an artery to dilate after an applied stress or pressure to the vessel constricts it. Individuals with T2DM following a dietary pattern that was low in glycemic load (high in fiber, low in fat) were shown to have a greater FMD than those not following this type of dietary pattern; presumably this effect was related to greater NO availability[16].

Elevated blood lipids contribute to changes in the structure and function of blood vessels. Atherosclerosis, or hardening of the blood vessels, may result from hyperlipidemia, reducing the compliance and function of the blood vessel. Hyperlipidemia also contributes to oxidative stress, causing inflammation[26]. The DASH dietary pattern has been shown to promote healthy blood lipid levels[16, 18, 30]. Patients with T2DM given counseling on a diet similar to the DASH dietary pattern have been shown to have a 19% relative reduction in LDL cholesterol while HDL cholesterol remained constant when compared to controls with no diet therapy[18]. In another study, consumption of a DASH-style diet was related to a greater improvement in triglyceride levels among those with hypertriglyceridemia[24]. A similar study that includes a reduction of refined and processed foods and sugar sweetened beverages, yielded similar results in post-menopausal women with T2DM[19]. According to a review, when whole grains are considered alone, those who consume more tended to have lower concentrations of total and LDL-cholesterol, as well as lower fasting blood glucose, indicating an improved lipid profile in addition to improved blood glucose control[40]. Given that the DASH dietary pattern is high in whole grains, positive effects on blood lipids would be expected with greater compliance to a DASH-style diet.

Empirical evidence has shown that chronic inflammation in an otherwise healthy individual can have adverse cardiovascular effects, particularly in those with T2DM. Inflammation can lead to hypertension, oxidative stress, which can damage the structure and function of the endothelium, and if left untreated, cause morbidity and mortality[2]. The DASH dietary pattern encourages healthy fats including olive oil, which contains the monounsaturated fatty acid oleic acid. Olive oil as well as many fruits, vegetables, and legumes has a high polyphenol content that helps reduce oxidative stress and offers benefits relative to endothelial dysfunction, hypertension, platelet aggregation, diabetes, and inflammation[30]. Antioxidant polyphenols and vitamin C in the DASH diet have been shown to reduce oxidative stress[3]. Increased production of reactive oxygen species, decreased production of nitric oxide, and decreased bioavailability of antioxidants has been associated with hypertension, cardiovascular disease and related mortality[32]. The high polyphenol content and the high nitrate content associated with some fruits and vegetables is associated with decreasing the presence of reactive oxygen species and increasing the bioavailability of nitric oxide in order to improve vasodilation and reduce vascular stress[32]. Sodium, which is restricted on the DASH diet, has been shown to increase markers of oxidative stress and decrease antioxidant capacity[2]. In response to consumption of a dietary pattern similar to the DASH dietary pattern, the inflammatory marker homocysteine was shown to decrease, indicating that inflammation may decrease as well on a DASH-style diet[18].

The DASH dietary pattern has been empirically shown to reduce hypertension; however the aforementioned research suggests that compliance to this dietary pattern may have broader benefits to vascular structure and function. The inherent components of the DASH dietary pattern have been shown to independently promote vasodilation and NO production[7,18,20]. Individuals who follow a DASH dietary pattern also have improved metabolic parameters including blood lipids and glucose over those who do not[18, 20, 24, 30]. Chronic inflammation has the potential to negatively impact blood vessel structure and function. Greater adherence to a DASH dietary pattern has been related to reduced markers of inflammation[2,7,18,30]. While there is a lack of randomized clinical trial evidence to support the benefits of the DASH dietary pattern and measured changes to vascular health, it stands to reason that eating a DASH dietary pattern will have beneficial effects to the structure and function of the vasculature.

D. Gaps in the Literature

Diets that emphasize fruits, vegetables, low-fat dairy, whole grains, and that are low in red meat are likely to be associated with a positive change in vascular structure and function. However, to date there is a lack of empirical evidence demonstrating that this type of dietary pattern (e.g. the DASH dietary pattern) is related to vascular health. Given the potential benefits that components of this diet can have on the vascular endothelium, studies that focus on the effects of this dietary pattern on measures of vascular structure and function are needed. Positive results from these studies would help inform future dietary intervention trials aimed at CVD prevention in T2DM.

Purpose

This study will examine whether adherence to a DASH-style diet is associated with measures of vascular health in individuals with T2DM. Our hypothesis is that there will be a positive relationship between adherence to a DASH-style diet and vascular structure and function in individuals with T2DM.

Methods

A. Study Population

The data for this study was collected from adolescents and young adults enrolled in a study designed to examine the effects of obesity and T2DM on cardiovascular structure and function. This is a secondary data analysis of findings from the T2DM CVD study and the methods have been previously published and are briefly described below[33]. The present study examined dietary data and vascular structure and function measures from a cohort of participants with T2DM (n=126). Adolescents and young adults with previously diagnosed T2DM were recruited from the Cincinnati Children's Hospital Diabetes Clinic. The average duration of diabetes in this population was equal to 3.6±2.6 years.

Written informed consent was acquired prior to the study from patients 18 years and older or the parent or guardian for patients younger than 18 years. For those participants under 18 years, written assent was obtained according to the guidelines established by the institutional review board at Cincinnati Children's Hospital Medical Center.

B. Data Collection

This is a cross-sectional study. Participants were asked to fast for a minimum of 10 hours overnight. All assessment visits were completed in the CCHMC diabetes clinic and study assessments included a demographics and clinical characteristics questionnaire, and the collection of anthropometric, BP, laboratory, and arterial stiffness measures as described below. Dietary intake data were collected within 2 weeks of the clinical assessment visit by telephone.

Anthropometrics:

Using a calibrated stadiometer, trained personnel recorded 2 measures of height with the patient standing wearing socks with heels together and toes at a 45° angle and the head in the Frankfort horizontal plane. A third measurement was taken if the first two readings were >0.5 cm apart. Using a Health-o-meter electronic scale dedicated exclusively to this study, weight was measured twice. A third measurement was taken if the first two were >0.3 kg. BMI was calculated as kilograms per meters squared and adjusted for participant height and weight. BMI z-score was calculated for those under 18 and for those over 18, z-scores for an 18 year old was used.

Blood Pressure:

Using the standardized protocol according to the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents[36], BP was measured with a mercury sphygmomanometer by trained personnel. BP examiners were recertified annually by receiving 16 hours of instruction and evaluation. Based on arm circumference, the appropriate cuff was selected and placed around the upper arm of the participant while they were sitting with feet resting on a flat surface and their right arm resting at heart level. Three measurements were taken by rapidly inflating the cuff to the maximum inflation level and deflating at a rate of 2 mm Hg/s, with a 60 second rest between each measurement, where the pulse rate was measured for 30-60 seconds. The first Korotkoff phase (K1) was determined at the first event of two consecutive beats; K4 was determined when the sounds became muffled and K5 was when the sounds disappeared. The 3 measurements were then averaged to find the mean systolic and diastolic BP. If 2 of the 3 readings varied by >10 mm Hg, a fourth reading was performed and included in the average.

Physical Activity:

The Actical™ accelerometer was worn on the waist of the participant during waking hours for a 7 day period to assess physical activity. This tool can count movement in all directions. From these data, activity counts per minute were calculated and averaged over 7 days.

Biochemical Measures:

The Hitachi model 704 glucose analyzer measured fasting plasma glucose with intra-assay and interassay coefficients of variation of 1.2% and 1.6% respectively. Plasma insulin was measured by radioimmunoassay with an anti-insulin serum labeled 125I raised in guinea pigs, as well as a double antibody method to distinguish bound from free tracer. This has a sensitivity of 2 pmol using intra-assay and inter-assay coefficients of variation of 5% and 8% respectively. Fasting plasma lipid profile assays were completed in a laboratory that was standardized by the National Heart, Lung, and Blood Institute and Centers for Disease Control and Prevention, using the Friedewald equation[23] to calculate low-density lipoprotein (LDL) cholesterol concentration. Non-HDL values were obtained by subtracting HDL cholesterol values from total cholesterol values to give a better indication of the lipid profile including remnant particles. High sensitivity C-reactive protein (CRP) was measured with a high sensitivity enzyme linked immunoabsorbant assay. The HbA1c content of red blood cells was measured with high-performance liquid chromotagraphy.

Dietary Intake:

Dietary intake data were collected randomly using three 24-hour recalls over a two week time period. This information was collected within two weeks of the patients' in-clinic assessment visit. The telephone interviews were performed by trained dietitians from the Cincinnati Center for Nutrition Research (CCNR) at CCHMC. Two dimensional food models were provided to the participants to assist with portion recall. Three day dietary data were analyzed for nutrient content using the Minnesota Nutrient Data Systems (NDS) software (2012). A DASH Score[14] was calculated using the data of averages for nutrients and food servings from three 24-hour dietary recalls. This score was calculated as the sum of 9 component scores based on daily intake of grains, vegetables, fruits, dairy, meat/poultry/fish/eggs, nuts/seeds/legumes, fats/oils, sweets and sodium. To calculate the component scores, the adolescent's intake was compared against food goals established according to age, gender, and energy level in the Dietary Guidelines for Americans[35], and the DASH Collaborative Research Group[17]. A sedentary activity level was used for these calculations based on the physical activity recommendations from the Institute of Medicine[6]. A maximum DASH score for each component was 10, which was achieved by meeting the food intake recommendation, whereas lower intakes were scored proportionately. The grain and dairy component scores were divided into 2 components: total grains and whole grains and total dairy and low-fat dairy. For each of these components, the maximum score was 5. An overall DASH score was calculated, which ranged between 0 and 90, with a higher score indicating a higher diet quality.

C. Carotid Ultrasonography

A registered vascular technologist performed the carotid ultrasonography studies with high-resolution B-mode ultrasonography and a high-resolution linear array vascular transducer. A 2-

dimensional image was obtained of the carotid artery from the far wall to measure intima media thickness in the common, bifurcation (bulb), and internal segments. To calculate arterial stiffness, images of the common carotid with near and far wall were obtained for M-mode evaluation of peak and minimal diameters. The Camtronic Medical System software was used to read the digital images and carotid stiffness was calculated based on the Peterson's elastic modulus (PEM)[27] and Young's elastic modulus (YEM)[8]. Because pulse wave amplification along the arterial tree causes an overestimation of the brachial systolic BP, the SphygmoCor device was used to measure central BPs to use in the carotid stiffness calculations. On average, the central BP readings were obtained no more than 30 minutes before the carotid ultrasound.

D. Arterial Stiffness Measurements

Each vascular measurement was taken 3 times after the participant was in the supine position for 5 minutes and averaged to use in the analyses. A DynaPulse Pathway instrument was used to measure BrachD. A standard cuff sphygmomanometer obtained brachial artery pressure curves from arterial pressure signals, assuming a straight tube brachial artery and T-tube aortic system.

A SphygmoCor SCOR-PVx System was used to measure carotid-femoral PWV and AI by applying a tonometer on the artery in question to obtain electro-cardiographic (ECG)-gated pressure data. PWV is calculated as the difference in the carotid-to-femoral path length that is measured directly divided by the difference in the R wave from the ECG to the foot of the pressure wave taken from the superimposed ECG and pressure tracings. For AI, the pressure waves are calibrated using mean arterial pressure (MAP) and the diastolic BP from the same arm. To estimate the central aortic pressure tracing and calculate AI, a validated generalized transfer function is used. Values are adjusted to a heart rate (HR) of 75 beats per minute since AI is affected by HR.

E. Statistical Analysis

All analyses were performed with Statistical Analysis Software (SAS Institute Inc, version 9.2, Cary, NC). Means and standard deviation (SD) were determined for continuous measures and frequency data was generated for categorical measures. Distribution of the data was assessed to determine normality and log transformed if the data did not have a normal distribution. Simple Pearson's correlations were derived for DASH score and demographics, clinical, anthropometric, and vascular structure and function measures. Relationships between DASH score and vascular structure and function were determined using the general linear model (GLM) procedure in SAS adjusting for the following covariates (determined a priori): age, gender, race, BMI z-score, duration of diabetes, HbA1c, daily calorie intake, and activity level. Significance was determined as $p<0.05$.

Results

Population characteristics are displayed in **Table 1**. The mean age of participants was 22.9 ± 3.8 years, and the sample population consisted primarily of Caucasian females. The duration of T2DM was an average of 8 years and the BMI z-score indicated that the participants were overweight or obese on average. Mean diastolic blood pressure was within normal limits, but systolic blood pressure was slightly elevated based on standards established by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure[36]. Based on established standards[38], normal limits for fasting insulin, fasting glucose, and HbA1c should be <25 uIU/mL, <70mg/dL, and <6.5%, respectively. As compared to these values in this study cohor, mean insulin values were within normal limits, however mean fasting glucose and HbA1c levels were above normal, indicating that blood glucose was not under control (**Table 1**). Average triglyceride and non-HDL cholesterol levels were within the normal range in this population[29,38]. Average DASH adherence in this population was low as reflected by a mean DASH score that was 39% of the highest possible score. Calorie intake was low, as compared to the calorie intake for an 18 year old female with a sedentary activity level[38]. Mean physical activity was also low in this population, based on a moderate activity level for this group, which is 1500 counts per minute[11].

Average vascular measures can also be found in **Table 1.** The mean augmentation index (AI) was 6.27%, brachial distensibility (BrachD) was 5.22% and pulse wave velocity (PWV) was 7.02m/s. When compared to these same measures in a healthy population of similar age, the average AI and PWV were higher while average BrachD was lower[33] indicating stiffer vessels and lower vascular distensibility in this population. Also compared to a healthy population, measures of internal carotid intima media thickness (cIMT) value were within the expected range, however the bulb and common cIMT were greater, indicating thicker vessels[33]. C-reactive protein levels were high in this population, as compared to established standards[38].

Table 1. Population Characteristics			
T2DM (n=126)			
Variable	**N**	**Mean or %**	**SD**
Demographics			
Age (years)	126	22.9	3.8
Race (% non-white)	125	33.60%	
Sex (% Male)	125	31.20%	
Clinical and Anthropometric			
Duration T2DM (years)	126	8.0	3.3
BMI Z-Score	126	1.96	0.61
Systolic Blood Pressure (mm Hg)	126	121	13.8
Diastolic Blood Pressure (mm Hg)	126	76	10.1
Fasting Insulin (µU/mL)	63	22.2	15.0
Log Fasting Glucose (mg/dL)	120	202.1	122.7
Log Triglycerides (mg/dL)	114	132.7	79.4
Non-HDL (total - HDL) (mg/dL)	115	130.3	45.1
HbA1c (%)	119	8.6	3.2
CRP (mg/L)	116	5.3	4.7
Dietary Data and Intake			
DASH Score	125	35.2	8.0
Daily Calorie Intake (kcal/day)	110	1789	611.8
Physical Activity (Counts per Minute)	95	222.1	135.0
Vascular Measures - Function			
Augmentation Index (%)	115	6.3	12.5
Brachial Distensibility (mm)	96	5.2	0.9
Pulse Wave Velocity (m/s)	109	7.0	1.3
Vascular Measures - Structure			
Internal cIMT (mm)	116	0.5	0.1
Common cIMT (mm)	126	0.5	0.1

Bulb (Bifurcation) cIMT (mm)	125	0.6	0.2
Footnote: T2DM=type 2 diabetes mellitus, BMI-body mass index, HDL=high density lipoprotein, HbA1c=hemoglobin A1c, CRP=c-reactive protein, DASH=dietary approaches to stop hypertension, N=number of subjects, SD=standard deviation, cIMT=carotid intima media thickness			

Correlations between the DASH score and the study covariates are displayed in **Table 2**. Daily calorie intake was negatively correlated with DASH score (p<0.0001). All other covariates were not significantly correlated, however there were several trends noted. There was a trend for a positive relationship between duration of diabetes and DASH score, (P<0.07), indicating that greater DASH adherence was related to longer duration of diabetes. Conversely, there was a trend for a negative relationship between fasting glucose and common cIMT and DASH score (P<0.9 and P=0.10, respectively), indicating that as DASH adherence increased, both fasting glucose and common cIMT decreased.

Table 2. Correlations of Covariates with DASH Score

Vascular Measure	DASH Score		
	N	**r**	**p-value**
Demographics			
Age (years)	125	-0.06	0.53
Clinical and Anthropometric			
Duration T2DM (years)	125	0.16	0.07
BMI Z-Score	125	0.05	0.59
Systolic Blood Pressure (mm Hg)	125	-0.12	0.16
Diastolic Blood Pressure (mm Hg)	125	-0.11	0.21
Fasting Insulin (uIU/mL)	62	0.04	0.76
Log Fasting Glucose (mg/dL)	119	-0.16	0.09
Log Triglycerides (mg/dL)	113	0.11	0.25
Non-HDL (total-HDL) (mg/dL)	114	0.07	0.48
HbA1c (%)	118	-0.10	0.28
CRP (mg/L)	115	-0.01	0.91
Dietary Data and Intake			
Daily Calorie Intake (kcal/day)	110	-0.6	<0.0001
Physical Activity (Counts per Minute)	94	0.02	0.84
Vascular Measures - Function			
Augmentation Index (%)	114	0.10	0.29
Brachial Distensibility (mm)	95	0.07	0.49
Pulse Wave Velocity (m/s)	108	-0.01	0.94

Vascular Measures - Structure			
Internal cIMT (mm)	115	-0.10	0.27
Common cIMT (mm)	125	-0.15	0.11
Bulb (Bifurcation) cIMT (mm)	124	-0.11	0.22
Composite cIMT (mm)	125	-0.11	0.22
Footnote: DASH=dietary approaches to stop hypertension, N=number of subjects, r=Pearson product moment correlation coefficient, p<0.05, T2DM=type 2 diabetes mellitus, BMI=body mass index, HDL=high density lipoprotein, HbA1c=hemoglobin A1c, CRP=c-reactive protein, cIMT=carotid intima media thickness			

Tables 3 and 4 show the GLM results for DASH score and clinical risk factors and vascular structure and function. All models were adjusted for BMI z-score, gender, race, duration of diabetes, HbA1c, total calorie intake, and physical activity (METs/day). Model results show effect estimates in the expected direction; however there were no significant relationships, suggesting that after adjusting for the possible influences of confounders, DASH adherence was not associated with clinical or vascular measures in individuals with T2DM.

Table 3. General Linear Model with Clinical Risk Factors			
	DASH Score		
Clinical Measures	**Estimate**	**SE**	**p-value**
Systolic Blood Pressure	0.02	0.21	0.94
Diastolic Blood Pressure	0.02	0.16	0.90
Fasting Glucose	-0.007	0.01	0.20
Fasting Insulin	0.005	0.01	0.73
Triglycerides	0.009	0.01	0.32
Non-HDL Cholesterol	0.63	0.72	0.39
CRP	0.002	0.31	0.94
Footnote: All measures in the model were adjusted for the following covariates: BMI z-score, gender, race, duration of diabetes, HbA1c, total calorie intake, physical acitivity (counts per minute); DASH=dietary approaches to stop hypertension, SE=standard error, p<0.05, HDL=high density lipoprotein, CRP=c-reactive protein			

Table 4. General Linear Model with Vascular Structure and Function			
	DASH Score		
Vascular Measures	**Estimate**	**SE**	**p-value**
Augmentation Index	-0.18	0.22	0.42

Brachial Distensibility	0.003	0.003	0.42
Pulse Wave Velocity	-0.002	0.003	0.46
cIMT Internal	0.004	0.004	0.35
cIMT Common	-0.001	0.003	0.73
cIMT Bulb	-0.001	0.004	0.78
Footnote: All measures in model were adjusted for the following covariates: BMI z-score, gender, race, duration of diabetes, HbA1c, total calorie intake, physical activity (counts per minute); DASH=dietary approaches to stop hypertension, SE=standard error, p<0.05, cIMT=carotid intima media thickness			

Discussion

The aim of this study was to examine the relationship between adherence to a DASH dietary pattern and vascular structure and function in individuals with T2DM. Average blood pressure for these individuals was within the normal range for diastolic pressure, however the average systolic values were bordering on pre-hypertension. In this sample, clinical measures such as glucose and HbA1c indicated that average blood sugar levels were not under control. DASH dietary adherence and physical activity were low compared to reports from healthy individuals of the same age[38]. Both vascular structure and function measures suggested that the study cohort had stiffer blood vessels on average, compared to a healthy population[38]. However, there were no significant associations between DASH dietary adherence and any of the vascular structure and function measures assessed in this study after adjusting for potential confounding factors. This finding was contrary to our hypothesis.

The beta estimate determined from the GLM procedure gives a measure of the size and direction of the effect of the independent variable (in this study, DASH dietary adherence as measure by DASH score) on each dependent variable (in this study, vascular structure and function). While there were no significant associations between the DASH score and vascular outcomes in this study, the beta estimates were in the expected direction, based on previous research. For example, the estimates for DASH adherence with AI, PWV, and common and bulb cIMT were negative, and with BrachD were positive, albeit not significantly so in this population. These results are suggestive of vascular benefits of a DASH-type diet and support findings of others[2, 7, 18, 30, 37]. Importantly for future studies, the standard errors of the vascular measures in our population were either equal to or greater than the estimates in our cohort. This suggests a high level of variability in the vascular outcome measures in this study and the need for a larger sample to allow significant associations to be identified.

Individuals with T2DM are at a higher risk for structural and functional vascular abnormalities[9,14,16,20,30,33,34], leading to consequences such as stroke, CVD, and mortality[9]. This may occur as a consequence of elevated blood pressure[18, 21] dyslipidemia[12, 13] and chronic inflammation[22, 30]. Recent studies suggest a relationship between increased arterial thickness and poor glycemic control in individuals with diabetes[10, 39]. In the present study, average measures of bulb and common cIMT were greater than those reported in previous publications from healthy adults[33]. Likewise, mean fasting glucose and HbA1c were outside normal limits suggesting poor glycemic control on average in this population. Notably, there was a trend for a negative relationship between DASH score and fasting glucose in this study suggesting benefits of a DASH dietary pattern on glycemic control in young adults with T2DM. This association was attenuated in fully adjusted models, although the beta estimate was in the expected direction. These associations reinforce the need for larger studies to examine whether a DASH type diet may have clinical benefits to individuals with T2DM.

There are limitations to the present study worth noting. The samples size was relatively small and, given the variance in the outcomes measures as previously noted, would need to be increased substantially in order for the power to be viable to detect significant associations. When compared to other studies assessing the same vascular outcomes, sample sizes were 4 to 5 times larger[33]. The present study was cross-sectional, so causality could not be determined. This was also a secondary data analysis using data derived from a study designed to examine the relationship between T2DM and obesity with vascular outcomes. The design did not emphasize recruitment of individuals with heterogeneous DASH dietary adherence, and as a consequence, DASH scores in the sample were skewed to the left (low adherence). While this was reflective of typical dietary intakes in this population, this may have contributed to the null findings in this study. The covariates tested were factors known a priori to be associated with vascular structure and function; however our regression analyses may not have adjusted for all confounders that could have influenced the results, e.g., income[13]. Also, we controlled for duration of diabetes in the analysis, however it is difficult to control for compliance to diabetes self-management, which may have impacted the relationship between DASH dietary adherence and vascular health indices. For example, the patients may have restricted food sources of carbohydrates in their diet, many of which are food components of the DASH dietary pattern (fruits, vegetables, whole grain breads, milk), contributing to the low DASH adherence and the inability to detect significant associations. Lastly, dietary recall was the measure used to determine DASH dietary adherence, and there are inherent limitations in this dietary assessment method such as recall bias and erroneous portion estimation.

A great strength of this study, however, was that all patients were diagnosed with T2DM and were being treated at the same clinic at CCHMC, meaning they were receiving similar treatment regimens and counseling techniques for the duration of their diabetes management. Also, a nationally recognized expert in the field of vascular research[33, 34] was responsible for the measurement of vascular structure and function in this study. As a result, there were great quality control measures put in place relative to the outcome measures, such as experienced nurses and technicians taking the measurements, well maintained instruments, and quality control associated with the lab analyses. Lastly, this study follows a unique population of young adults with T2DM that has yet to be assessed for DASH dietary adherence as it relates to vascular health.

Our data do not support the hypothesis that the DASH dietary pattern is related to vascular structure and function, however due to the limitations of the study, more research needs to be done. There is a need for larger studies, as evidenced by the variability of the outcome measures. There were unadjusted trends in this study that indicate that the DASH dietary pattern may improve fasting glucose levels as well as internal cIMT. Large, randomized clinical trials would provide the strongest evidence of a favorable impact of a DASH dietary pattern on vascular health in individuals with T2DM.

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